

## **FIG.\_ 1A**

**SEQ ID NO: 1**

**Nucleotide Sequence Tankyrase Homologue isotype1**

CTTGAAAGACACTGGATTCATACTTTGCCTGGGTTATCTCTGTGTCACTACATAGACAAATA  
TTAGCTGTGAGCAGATCTTTTGTGCTTAGTCCCCCAGTTAGCAGAACATTCTGTGAGA  
TAGATGTGGAAAGGAATTCTAGCAAGAGTTGTCACTGTATCATAAGGTTGTGATTACATATTAA  
GTTTATACTTGAACATCTGAAAATGTATACATACTAAATATGCAGAACTCTATTGTAGAGTGAGAAA  
CATTTGAACCTTGAGCTTCAGTCACTTATTGTATTCTTCTTGAGGTTAGCAGTAGTACCAACCCA  
AGGCAC TGCTTAGGTACCTGCTGCTTAGTGGAGAGTCCCTGCGTTATCATTAAAGGTTGGCG  
GAAAGACGTAGTTGAATATTGCTTCAGAATGGTGCAGTGTCCAAGCACGTGATGATGGGGCCTTAT  
TCCTCTCATATGCATGCTCTTGGTCACTGCTGAAGTAGTCAATCTCCTTGCACATGGTGCAGA  
CCCCAATGCTCGAGATAATTGGAATTATACTCCTCTCCATGAAGCTGCAATTAAAGGAAAGATTGATGT  
TTGCATTGTGCTGTTACAGCATGGAGCTGAGCCAACCATCGAAATACAGATGGAAGGACAGCATTGGA  
TTTAGCAGATCCATCTGCCAAGCAGTGCTTACTGGTGAATATAAGAAAGATGAACTCTTAGAAAGTGC  
CAGGAGTGGCAATGAAGAAAAATGATGGCTACTCACACCATTAAATGTCAACTGCCACGCAAGTGA  
TGGCAGAAAGTCAACTCCATTACATTGGCAGCAGGATATAACAGAGTAAAGATTGTACAGCTGTTACT  
GCAACATGGAGCTGATGTCCATGCTAAAGATAAAGGTGATCTGGTACCATACACAATGCCTGTTCTTA  
TGGTCATTATGAAGTAACTGAACTTTGGTCAAGCATGGCCTGTGTAATGCAATGGACTTGTGGCA  
ATTCACTCCTCTCATGAGGCAGCTCTAAGAACAGGGTTGAAGTATGTTCTCTCTTAAGTTATGG  
TGCAGACCCAACACTGCTCAATTGTCACAATAAAAGTGCATAGACTGGCTCCCACACCACAGTTAA  
AGAAAGATTAGCATATGAATTAAAGGCCACTCGTTGCTGCAAGCTGCAAGGAGCTGATGTTACTCG  
AATCAAAAAACATCTCTCTGGAAATGGTAATTCAAGCATCCTCAAACACATGAAACAGCATTGCA  
TTGTGCTGCTGCATCTCCATATCCAAAAGAAAGCAAATATGTGAACTGTTGCTAAGAAAAGGAGCAAA  
CATCAATGAAAAGACTAAAGAATTCTTGACTCCTCTGCACGTGGCATCTGAGAAAGCTCATAATGATGT  
TGTGAAGTAGTGGTGAACATGAAGCAAAGGTTAATGCTCTGGATAATCTGGTCAGACTCTACA

CAGAGCTGCATATTGTGGTCATCTACAAACCTGCCGCCTACTCCTGAGCTATGGGTGTGATCCTAACAT  
TATATCCCTTCAGGGCTTACTGCTTACAGATGGAAATGAAAATGTACAGCAACTCCTCCAAGAGGG  
TATCTCATTAGGTAAATTAGGAGGCAGACAGACAATTGCTGGAAGCTGCAAAGGCTGGAGATGTCGAAAC  
TGTAAAAAAACTGTGTACTGTCAGAGTGTCAACTGCAGAGACATTGAAGGGCGTCAGTCTACACCCT  
TCATTTGCAGCTGGGTATAACAGAGTGTCCGTGGAATATCTGCTACAGCATGGAGCTGATGTGCA  
TGCTAAAGATAAAGGAGGCCTGTACCTTGACAAATGCATGTTCTATGGACATTATGAAGTTGCAGA  
ACTTCTGTTAACATGGAGCAGTAGTTAATGTAGCTGATTATGAAATTACACCTTACATGAAGC  
AGCAGCAAAGGAAAATATGAAATTGCAAACCTCTGCTCCAGCATGGTCAGACCCCTACCAAAAAAA  
CAGGGATGGAATACTCCTTGGATCTGTTAAAGATGGAGATACAGATATTCAAGATCTGCTTAGGG  
AGATGCAGCTTGCTAGATGCTGCCAAGAAGGGTTGTTAGCCAGAGTGAAGAAGTTGTCTTCTCCTGA  
TAATGTAATTGCCGCGATAACCAAGGCAGACATTCAACACCTTACATTAGCAGCTGGTTATAATAA  
TTTAGAAGTTGCAGAGTATTGTTACAACACGGAGCTGATGTGAATGCCAAGACAAAGGAGGACTTAT  
TCCTTACATAATGCAGCATCTACGGGCATGTAGATGTAGCAGCTCTACTAATAAGTATAATGCATG  
TGTCAATGCCACGGACAAATGGGCTTACACCTTGCACGAAGCAGCCAAAGGGACGAACACAGCT  
TTGTGCTTGTGCTAGCCATGGAGCTGACCCGACTCTAAAAATCAGGAAGGACAAACACCTTCTAGA  
TTTAGTTTCAGCGGATGATGTCAGCGCTCTGACAGCAGCCATGCCCATCTGCTCTGCCCTTTG  
TTACAAGCCTCAAGTGCCTAATGGTGTGAGAAGCCCAGGAGCCACTGCAGATGCTCTTCAGGTCC  
ATCTAGCCCATCAAGCCTTCTGCAGCCAGCAGCTTGACAACCTATCTGGAGTTTCAGAACTGTC  
TTCAGTAGTTAGTTCAAGTGGAACAGAGGGTGCTCCAGTTGGAGAAAAGGAGGTTCCAGGAGTAGA  
TTTAGCATAACTCAATTGTAAGGAATCTGGACTTGAGCACCTAATGGATATATTGAGAGAGAAC  
GATCACTTGGATGTATTAGTTGAGATGGGGCACAAGGAGCTGAAGGAGATTGAATCAATGCTTATGG  
ACATAGGCACAAACTAATTAAAGGAGTCGAGAGACTTATCTCCGGACAACAAGGTCTAACCCATATTT  
AACTTTGAACACCTCTGGTAGTGGAAACAATTCTTATAGATCTGTCCTGATGATAAAGAGTTTCAGTC  
TGTGGAGGAAGAGATGCAAAGTACAGTTGAGAGCACAGAGATGGAGGTATGCAGGTGGAATCTCAA  
CAGATACAATATTCTCAAGATTGAGGTTGTAACAAGAAACTATGGAAAGATAACACTCACCGGAG  
AAAAGAAGTTCTGAAGAAAACCACAACCATGCCAATGAACGAATGCTATTCTATGGGTCTCTTGT  
GAATGCAATTATCCACAAAGGCTTGATGAAAGGCATGCGTACATAGGTGTATTTGGAGGAGGTACTGGGTGTC  
TTATTTGCTGAAAACCTCTCCAAAAGCAATCAATATGTATATGGAATTGGAGGAGGTACTGGGTGTC  
AGTTCACAAAGACAGATCTGTTACATTGCCACAGGCAGCTGCTCTTGCCTGGTAACCTTGGAAA  
GTCTTCTGCAGTTGCAATGAAAATGGCACATTCTCCTCCAGGTATCACTCAGTCAGTCACTGGTAG  
GCCCAAGTGTAAATGGCCTAGCATTAGCTGAATATGTTATTTACAGAGGAGAACAGGCTTATCCTGAGTA  
TTAATTACTTACCAAGATTATGAGGCCTGAAGGTATGGTCATGGATAAATAGTTATTTAAGAAACTA  
ATTCCACTGAACCTAAATCATCAAAGCAGCAGTGGCCTACGTTACTCCTTGCTGAAAAAA  
AA

## FIG.\_ 1B

## **FIG.\_2A**

**SEQ ID NO: 2**

**Nucleotide Sequence Tankyrase Homologue isotype2**

CGCGCTGCTCCGCCGCCGGGGCAGCCGGGGCAGGGAGCCCAGCGAGGGCGCGGTGGCGCG  
CCCATGGGACTGCGCCGGATCCGGTGACAGCAGGGAGCCAAGCGGCCGGGCCCTGAGCGCGTCTCTC  
CGGGGGGCCTCGCCCTCCTGCTCGCGGGCCGGGCTCTGCTCCGGTTGCTGGCGCTTGTGGCTG  
TGGCGCGGCCAGGATCATGTCGGGTCGCCGCTGCCGGGGAGCGGCGTGCAGCGAGCGCCGG  
CCGAGGCCGTGGAGGCCGGCCCGAGAGCTGTTGAGGGCGTGCAGCAACGGGACGTGGAACGAGTCA  
AGAGGCCTGGTACGCCCTGAGAAGGTGAACAGCCGACACGGGGCAGGAATCCACCCGCTGCACT  
TCGCCGCAGGTTTGGCGAAAGACGTAGTTGAATATTGCTTCAGAATGGTCAAATGTCCAAGCAC  
GTGATGATGGGGCCTATTCTCTTCAATGCATGCTCTTGGTATGCTGAAGTAGTCAATCTCC  
TTTGCGACATGGCAGACCCCAATGCTGAGATAATTGAATTACTCCTCTCCATGAAGCTGCAA  
TTAAAGGAAAGATTGATGTTGCATTGTGCTGTTACAGCATGGAGCTGAGCAAACCATCCGAAATACAG  
ATGGAAGGACAGCATTGGATTAGCAGATCCATGCCAACAGCAGTGTACTGGTGAATATAAGGAAAG  
ATGAACCTTAGAAAGTCCAGGAGTGGCAATGAAGAAAAATGATGGCTCTACTCACACCATTAAATG  
TCAACTGCCACGCAAGTGTGGCAGAAAGTCAAACCTCATTACATTGGCAGCAGGATATAACAGAGTAA  
AGATTGTACAGCTGTTACTGCAACATGGAGCTGATGTCCATGCTAAAGATAAAGGTGATCTGGTACCAT  
TACACAATGCCCTTTATGGTCAATTGAAGTAATGAACTTTGGTCAAGCATGGCCTGTGAA  
ATGCAATGGACTTGTGGCAATTCACTCCTTCACTGAGGGCAGCTCTAAGAACAGGGTTGAAGTATGTT  
CTCTCTCTTAAGTTATGGCAGACCCAACACTGCTCAATTGTACAATAAAAGTGTATAGACTTGG  
CTCCCCACACCAGTTAAAGAAAGATTAGCATATGAATTAAAGGCCACTCGTTGCTGCAAGCTGCAC  
GAGAAGCTGATGTTACTCGAATAAAAACATCTCTGGAAATGGTGAATTCAAGCATCCTCAAA  
CACATGAAACAGCATTGCATTGTGCTGCATCTCCATATCCAAAAGAAAGCAAATATGTGAACGT  
TGCTAAGAAAAGGAGCAAACATCAATGAAAAGACTAAAGAATTCTGACTCCTCTGCACGTGGCATCTG  
AGAAAGCTCATAATGATGTTGAAGTAGTGGTAAACATGAAGCAAAGGTTAATGCTCTGGATAATC

TTGGTCAGACTTCTACACAGAGCTGCATATGTGGTCATCTACAAACCTGCCGCCTACTCCTGAGCT  
ATGGGGTGTGATCCTAACATTATATCCCTCAGGGCTTACTGCTTACAGATGGAAATGAAAATGTAC  
AGCAACTCCTCCAAGAGGGTATCTCATTAGTAATTAGGAGCAGACAGACAATTGCTGGAAGCTGAA  
AGGCTGGAGATGTCGAAACTGTAAAAAAACTGTGTACTGTTAGAGTGTCAACTGCAGAGACATTGAAG  
GGCGTCAGTCTACACCACTCATTTGCAGCTGGTATAACAGAGTGTCCGTGGTGGAAATATCTGCTAC  
AGCATGGAGCTGATGTGCATGCTAAAGATAAAGGAGGCCTGTACCTTGACAATGCATGTTCTTATG  
GACATTATGAAGTTGCAGAACTTCTTAAACATGGAGCAGTAGTTAATGTAGCTGATTATGGAAAT  
TTACACCTTACATGAAGCAGCAGCAAAAGAAAATATGAAATTGCAAACCTCTGCTCCAGCATGGTG  
CAGACCCCTACCAAAAAAAACAGGGATGAAATACTCCTTGGATCTGTTAAAGATGGAGATAAGATA  
TTCAAGATCTGCTTAGGGGAGATGCAGCTTGCTAGATGCTGCCAAGAAGGGTTGTTAGCCAGAGTGA  
AGAAGTTGTCTTCCTGATAATGTAATTGCGCGATAACCAAGGCAGACATTCAACACCTTACATT  
TAGCAGCTGGTTATAATAATTAGAAGTTGCAGAGTATTGTTACAACACGGAGCTGATGTGAATGCC  
AAGACAAAGGAGGACTTATCCTTACATAATGCAGCATCTACGGGATGCTAGATGTAGCAGCTCTAC  
TAATAAAGTATAATGCATGTGCAATGCCACGGACAAATGGCTTACACCTTGACAGCAGCCC  
AAAAGGGACGAACACAGCTTGTGCTTGTCTAGCCCATGGAGCTGACCCACTCTAAAAATCAGG  
AAGGACAAACACCTTGTAGATTAGTTAGCTCAGCGGATGTCAGCGCTCTGACAGCAGCCATGCC  
CATCTGCTCTGCCCTTGTACAAGCCTCAAGTGTCAATGGTGAGAAGGCCAGGAGCCACTGCAG  
ATGCTCTCTTCAAGGTCCATCTAGCCCATCAAGCCTTCTGCAGCCAGCAGTCTGACAACCTATCTG  
GGAGTTTCAGAACTGTCTCAGTAGTTAGTTCAAGTGGAACAGAGGGTGTCCAGTTGGAGAAAA  
AGGAGGTTCCAGGAGTAGATTAGCTAACTCAATTGTAAGGAATCTGGACTTGAGCACCTAATGG  
ATATATTGAGAGAGAACAGATCACTTGGATGTATTAGTTGAGATGGGCACAAGGAGCTGAAGGAGA  
TTGGAATCAATGCTTATGGACATAGGCACAAACTAATTAAAGGAGTCAGAGAGACTTATCTCCGGACAAC  
AAGGTCTTAACCCATATTTAACCTTGAACACCTCTGGTAGTGGAACAACTTATAGATCTGCTCCTG  
ATGATAAAGAGTTCAAGTGTGGAGGAAGAGATGCAAAGTACAGTTGAGAGCACAGAGATGGAGGTC  
ATGCAGGTGGAATCTCAACAGATAAACTTCAAGATTGAGAAGGTTGTAACAAGAAACTATGGG  
AAAGATAACACTCACCGGAGAAAAGAAGTTCTGAAGAAAACCACAACCATGCCAATGAACGAATGCTAT  
TTCATGGGTCTCTTGTGAATGCAATTATCCACAAAGGCTTGTAAAGGCATGCGTACATAGGTG  
GTATGTTGGAGCTGGCATTATTTGCTGAAAACCTTCCAAAGCAATCAATATGTATATGGAATTG  
GAGGAGGTACTGGGTGTCCAGTCACAAAGACAGATCTGTTACATTGCCACAGGAGCTGCTCTT  
GCCGGGTAACCTGGGAAAGTCTTCCGTCAAGTGCAGTTCAAGTGGAACATTCCTCCAGGTC  
ATCACTCAGTCAGTGTAGGCCAGTGTAAATGCCCTAGCATTAGCTGAATATGTTATTACAGAGGAG  
AACAGGCTTATCCTGAGTATTAATTACTTACAGATTAGGAGCCTGAAGGTATGGTCATGGATAAA  
TAGTTATTTAAGAAACTAATTCCACTGAACCTAAATCATCAAAGCAGCAGTGGCCTACGTTTAC  
TCCTTGCTGAAAAAAAAAA

## FIG.\_2B

**SEQ ID NO: 3**

**Amino Acid Sequence Tankyrase Homologue isotype1**

GFGRKDVEYLLQNGASVQARDDGGIPLHNACSGHAEVVNLLRHGADPNARDNWNYTPLHEAAIKG  
KIDVCIVLLQHGAEPITIRNTDGRTALDLADPSAKAVLTGEYKKDELLESARGNEEKMALLTPLNVNC  
HASDGRKSTPLHLAAGYNRVKIVQLLQHGADVHAKDKGDLVPLHNACSYGHYEVTELLVKHGACVNAM  
DLWQFTPPLHEAASKNRVEVCSLLLSYGADPTLLNCHNKSAILDAPTPQLKERLAYEFKGHSLLQAAREA  
DVTRIKKHLSEMVNFHPQTETALHCAAASPYPKRKQICELLRKGANINEKTKEFLTPLHVASEKA  
HNDVVEVVVKHEAKVNALDNLGQTSLHRAAYCGLQTCRLLLSYCDPNIISLQGFTALQMGNENVQQL  
LQEGISLGNSEADRQLLEAAKAGDVETVKKLCTVQSVNCRDIEGRQSTPLHFAAGYNRVSVVEYLLQHG  
ADVHAKDKGGLVPLHNACSYGHYEVAAELLVKHGAVVNADLWKFTPLHEAAAKGKYEICKLLLQHGADP  
TKKNRDGNTPLDLVKDGTDIQDLLRGDAALLDAAKKGCLARVKKLSSPDNVNCRDTQGRHSTPLHAA  
GYNNEVAEYLLQHGADVNAQDKGGIPLHNAASYGHVDVAALLIKYNACVNATDKWAFTPLHEAAQKG  
RTQLCALLLAHGADPTLKNQEGQTPLDVSADDVSALLTAAMPPSALPSCYKPQVLNGVRSPGATADAL  
SSGPSSPSSLASAASSLDNLGSFSELSSVSSSGTEGASSLEKKEVPGVDFSITQFVRNLGLEHLMDF  
EREQITLDVLVEMGHKELKEIGINAYGHRHKLIKGVVERLISGQQGLNPYLTLNNTSGSGTILIDLSPDDK  
EFQSVEEEMQSTVREHRDGGHAGGI FNRYNILKIQKVCKNLWERYTHRRKEVSEENHNHANERMLFHG  
SPFVNAAIHKGDERHAYIGGMFGAGIYFAENSSKSQNQYVYGGGTGCPVHKDRSCYICHRLQLLFCRV  
TLGKSFLQFSAMKMAHSPPGHHSVTGRPSVNGLALAELYRGEQAYPEYLITYQIMRPEGMVDG

**FIG.\_3**

**SEQ ID NO: 4**

**Amino Acid Sequence Tankyrase Homologue isotype2**

RCSARRGAAGGQGAQRGARVGAAHGTAPDPVTAGSQAARALSASSPGGLALLLAGPGLLLRLLLALLLAV  
AAARIMSGRRCAAGGAACASAAAEEAVEPAARELFECRNGDVERVKRLVTPEKVNSRDTAGRKSTPLHF  
AAGFGRKDVEYLLQNGANVQARDGGLIPLHNACSFHAEVNLLRHGADPNARDNWNYTPLHEAAI  
KGKIDVCIVLQLQHGAEPITRNTDGRTALDLADPSAKAVLTGEYKKDELLESARSGNEEKMMALLTPLNV  
NCHASDGRKSTPLHLAAGYNRVKIVQLLLQHGADVHAKDKGDLVPLHNACSYGHYEVTELLVKHGACVN  
AMDLWQFTPPLHEAASKNRVEVCSLLSYGADPTLLNCHNKSAILAPTPQLKERLAYEFKGHSLLQAAR  
EADVTRIKKHLSLEMVNFHKPQTHTETALHCAAASPYPKRQICELLRKGANINEKTKEFLTPLHVASE  
KAHNDVVEVVVKHEAKVNALDNLGQTSLHRAAYCGHLQTCRLLSYGCDPNIISLQGFTALQMGNEVQ  
QLLQEGLSLGNSEADRQLLEAAKAGDVETVKKLCVQSVNCRDIEGRQSTPLHFAAGYNRVSVEYLLQ  
HGADVHAKDKGGLVPLHNACSYGHYEVAEELLVKHGAVNVADLWKFPLHEAAAKGKYEICKLLLQHGA  
DPTKKNRDGNTPLDLVKDGTDIQDLLRGDAALLDAAKKGCLARVKKLSSPDNVNCRDTQGRHSTPLHL  
AAGYNNLEVAEYLLQHGADVNAQDKGGLIPLHNAAASYGHVDVAALLIKYNACVNATDKWAFTPPLHEAAQ  
KGRTQLCALLLAHGADPTLKNQEGQTPLDLSADDVSALLTAAMPPSALPSCYKPQVLNGVRSPGATAD  
ALSSGPSSPSSLASAASSLDNLGSFSSELSSVSSSGTEGASSLEKKEVPGVDFSITQFVRNLGLEHLM  
IFEREQITLDVLVEMGHKELKEIGINAYGHRHKLIKGVVERLISGQQGLNPYLTNTSGSGTILIDLSPD  
DKEFQSVEEEMQSTVREHRDGGHAGGI FNRYNILKIQKVCNKKLWERYTHRKEVSEENHNHANERMLF  
HGSPFVNAAIHKGFDERHAYIGGMFGAGIYFAENSSKSNSQYVYGGGTGCPVHKDRSCYICHRQLLFC  
RVTLGKSFLQFSAMKMAHSPPGHHSVTGRPSVNGLALAELYIYRGEQAYPEYLITYQIMRPEGMVDG

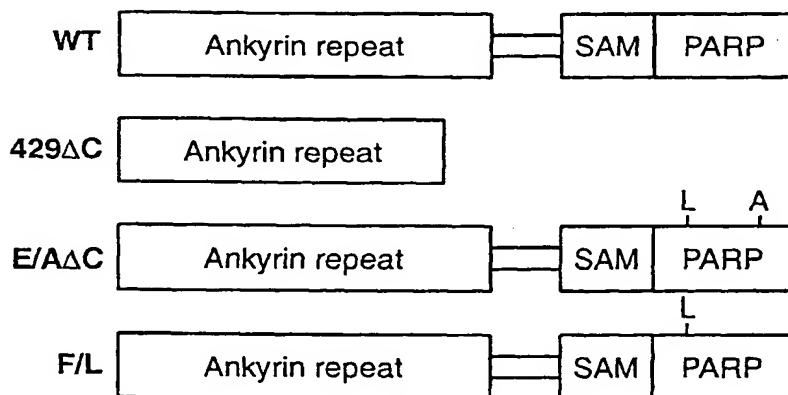
**FIG.\_4**

### Schematic Presentation of Dominant Negative Mutants for Tankyrase Homologue

#### Dominant Negative Mutants

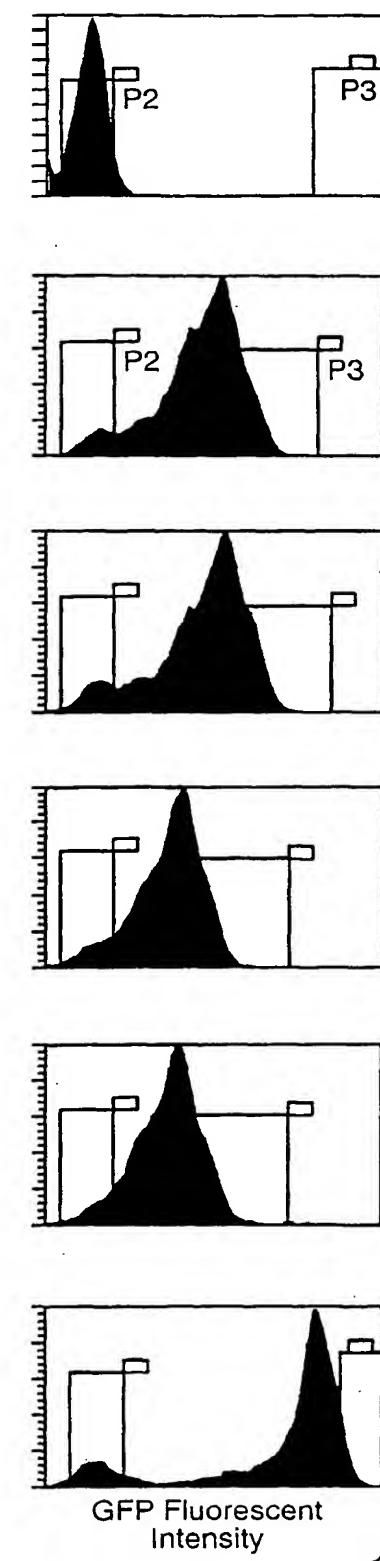
Truncation: 429 $\Delta$ C- of the C-terminal catalytic domain – truncation of the catalytic domain of PARP acts as a dominant negative when overexpressed *in vivo* (Oncogene 1999 Nov 25; 18(50):7010-5)

Point mutant: E945A $\Delta$ C- conserved residue in PARP domain, thought to be important in NAD<sup>+</sup> binding

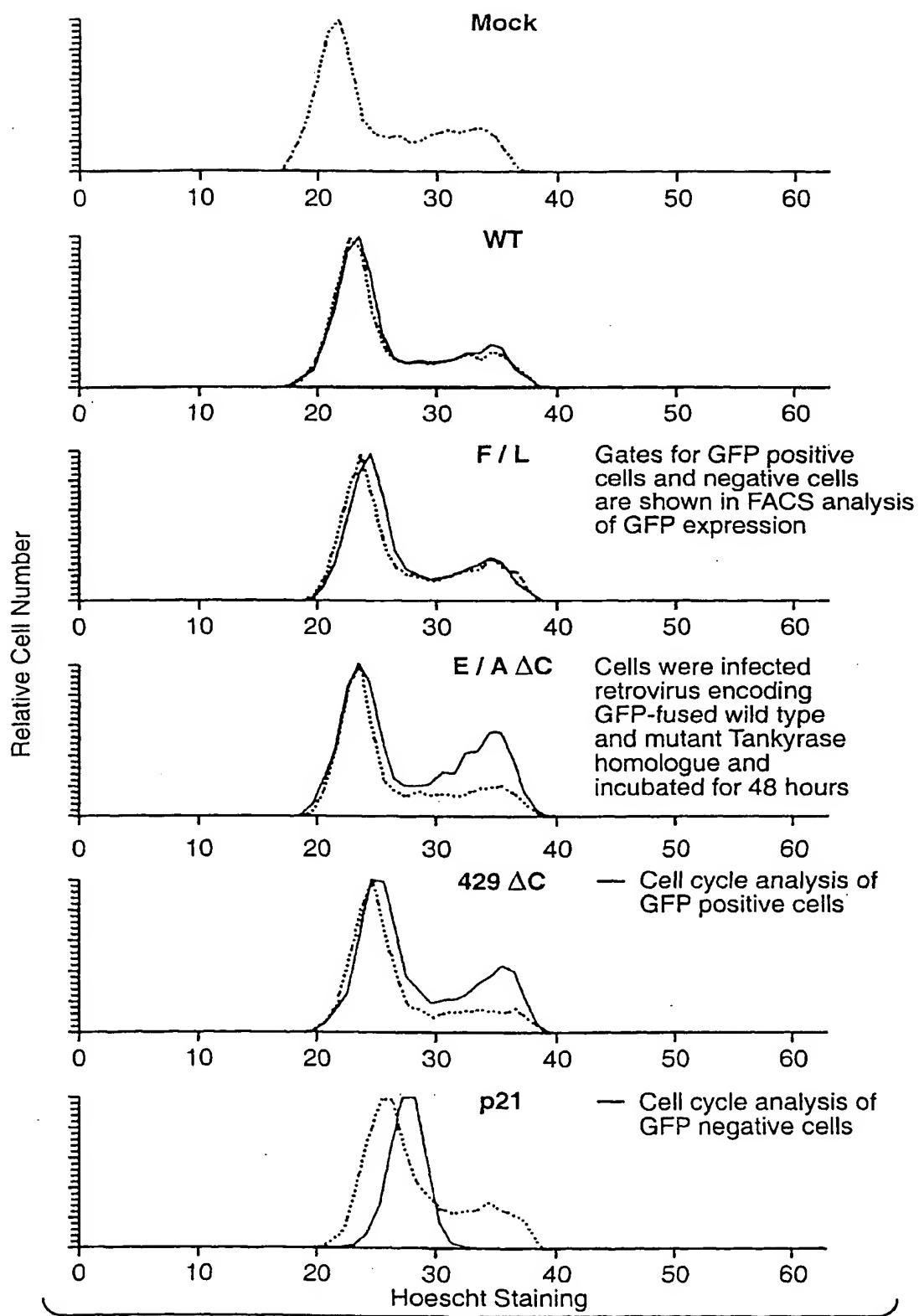


**FIG.\_5**

**Cell Cycle Analysis of A549 Cells  
Infected With GFP-fused Wild Type  
and Mutant Tankyrase Homologue**

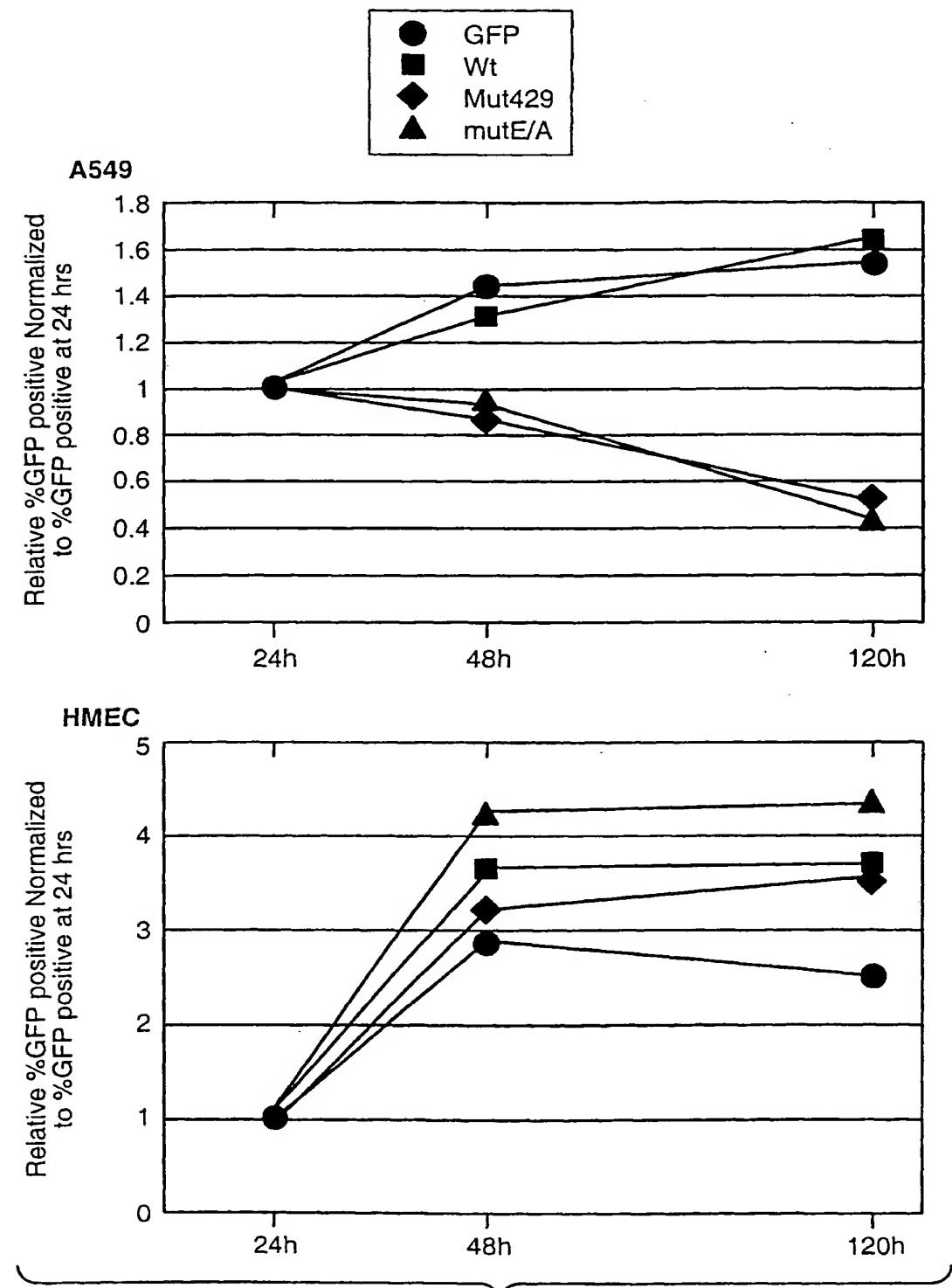


**FIG.\_6A**



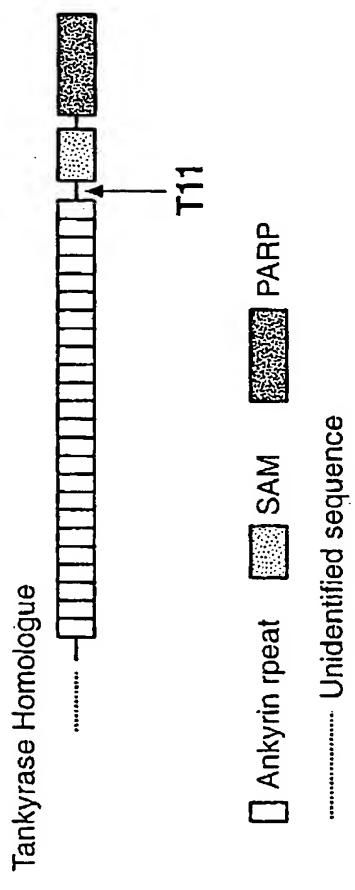
**FIG.\_6B**

**Kinetics of GFP Positive cells in A549 Cells and Human Mammary Epithelial Cells (HMEC) After Retrovirus Infection Encoding GFP-fused Wild Type and Mutant Tankyrase Homologue**



**FIG.\_7**

## The Binding Site of Antisense Oligos Against Tankyrase Homologue

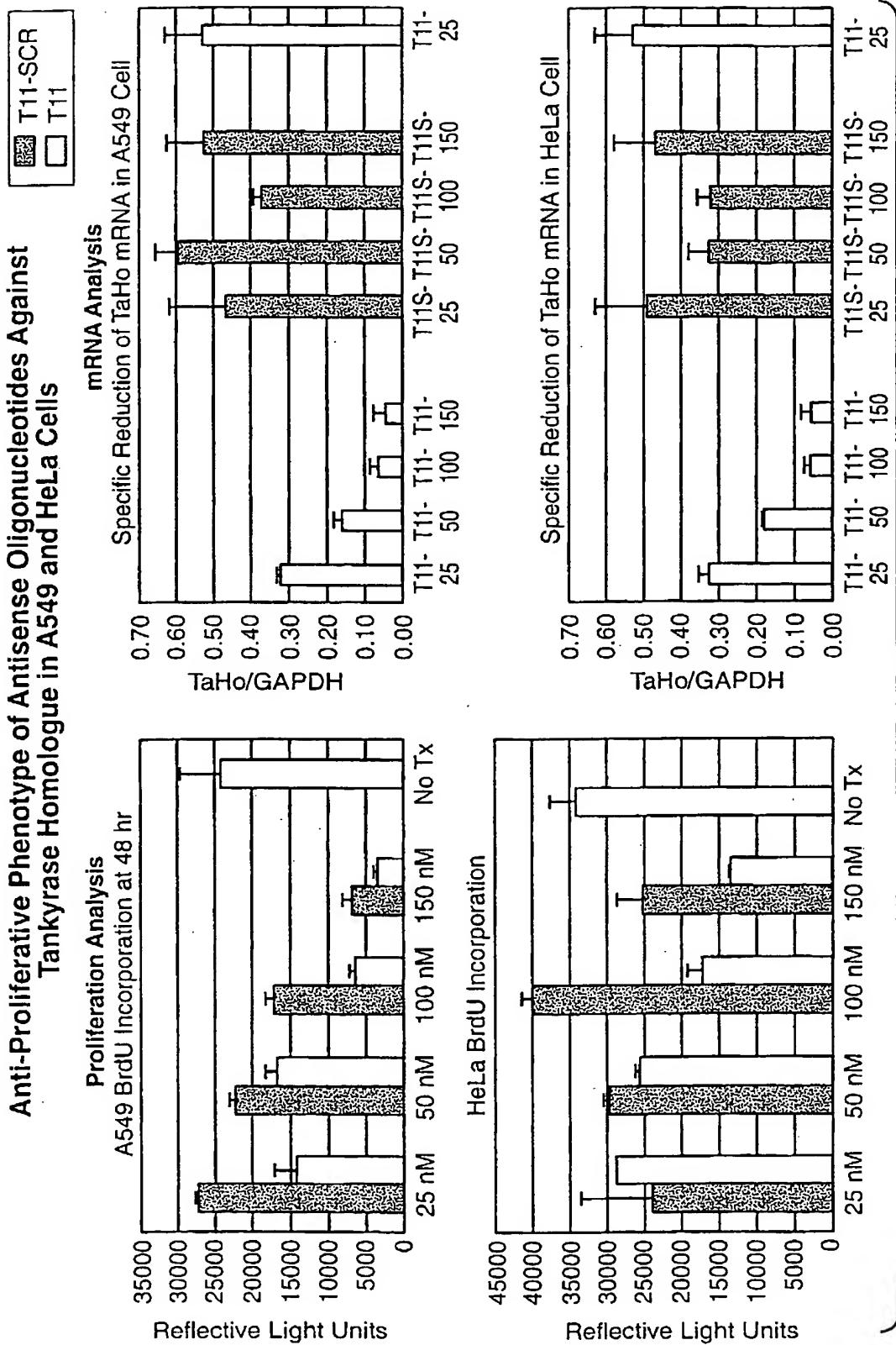


T11

Tankyrase Homologue	GTGGAACAGAGGGTGCCTCC	2838
Tankyrase	GTGGAACAGAGGGTGCCTCCAGTTGGAGAAAAGGAGGTCCAGGAGTAGATTAGCAT	
	ATGCCAGGGGATGGCGCGGGAAACAGAAAGGAAGGAGAAGTGTGGCTTGACAT	3091
	*** *	

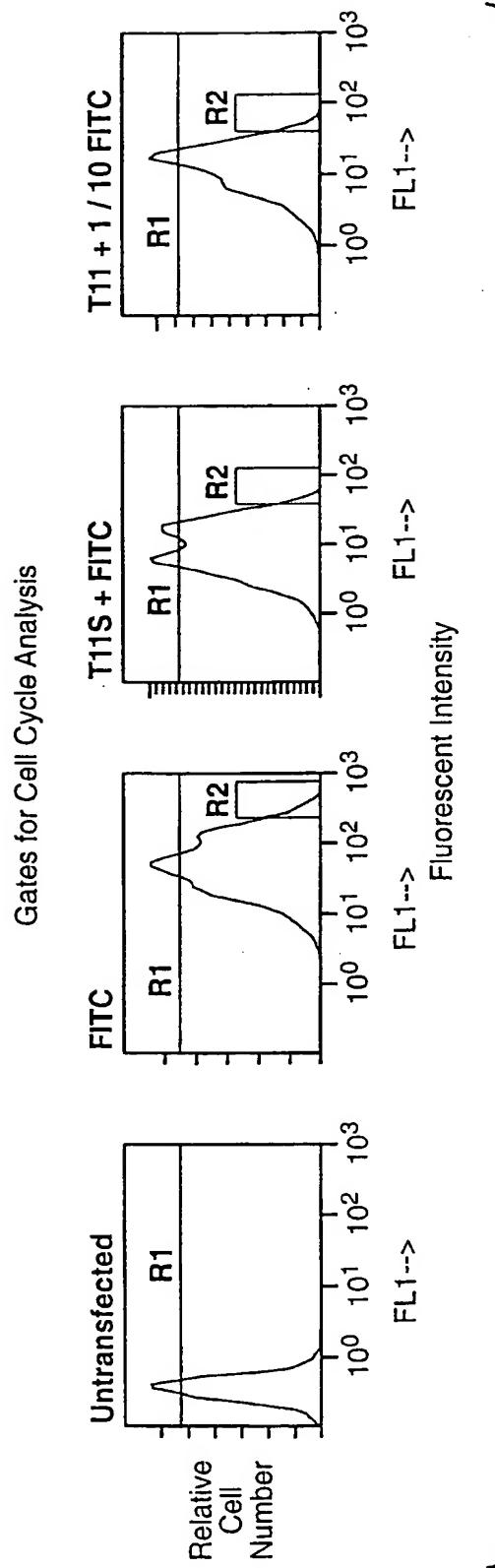
**FIG.-8**

**Anti-Proliferative Phenotype of Antisense Oligonucleotides Against  
Tankyrase Homologue in A549 and HeLa Cells**



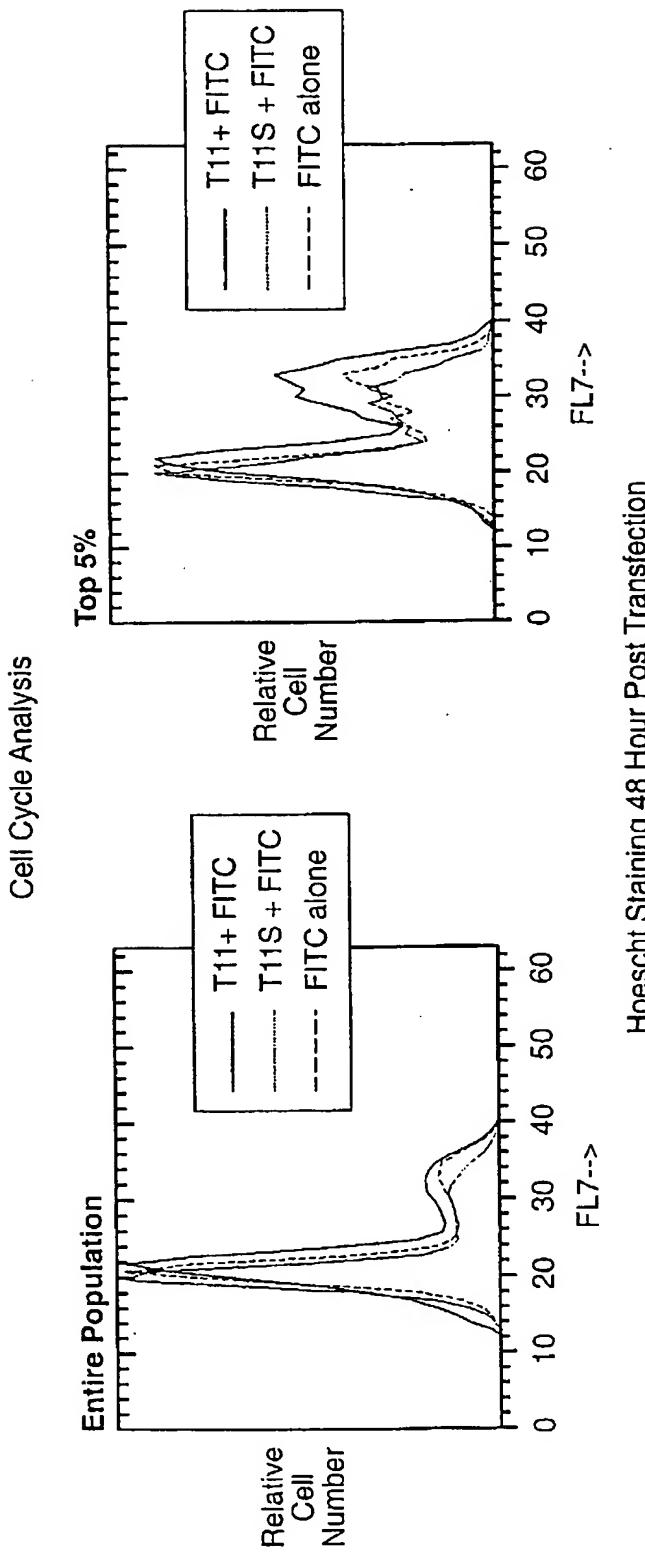
**FIG.\_9**

**Cell Cycle Analysis of A549 Cells Transfected with Antisense Oligonucleotides Against Tankyrase Homologue at 48 Hours, Antisense Oligonucleotides (T11) and Control Oligonucleotides (T11S) were transfected with FITC-labeled random 20mer Oligonucleotides (FITC), After 48 Hours, entire population (R1) and Top 5% (R2) of FITC transfected cells were analyzed for cell cycle**



**FIG. 10A**

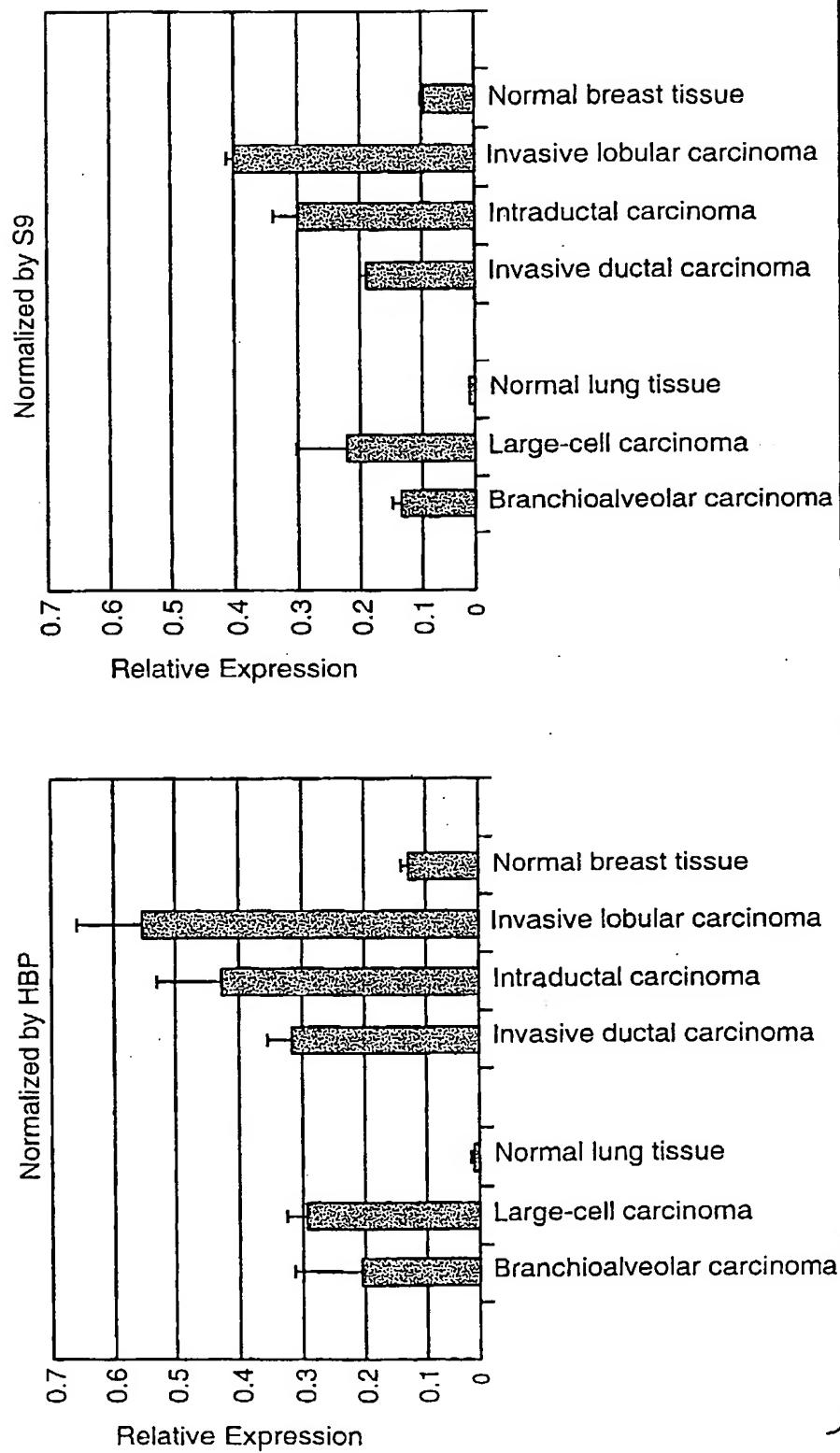
**Cell Cycle Analysis of A549 Cells Transfected with Antisense Oligonucleotides Against Tankyrase Homologue at 48 Hours, Antisense Oligonucleotides (T11) and Control Oligonucleotides (T11S) were transfected with FITC-labeled random 20mer Oligonucleotides (FITC), After 48 Hours, entire population (R1) and Top 5% (R2) of FITC transfected cells were analyzed for cell cycle**



**FIG. - 10B**

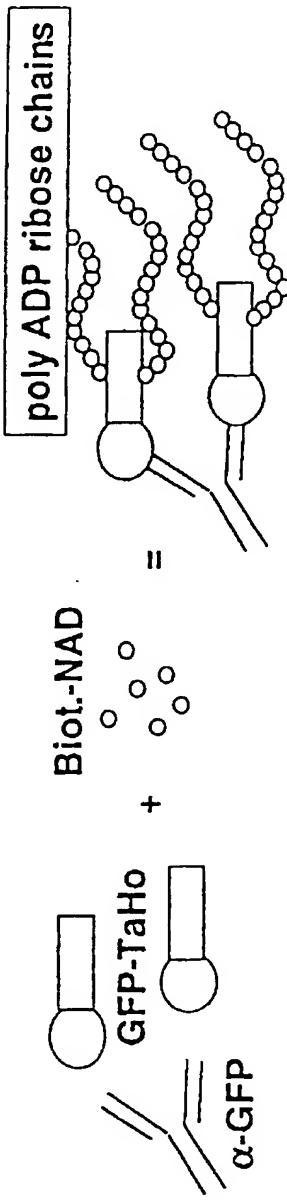
**FIG.- 11**

**mRNA Expression of Tankyrase Homologue in Several Tumors and Normal Tissues by a Taqman Analysis, mRNA Expression was Normalized by 90kDa Highly Basic Protein (HBP) and Ribosomal Protein S9 (S9)**



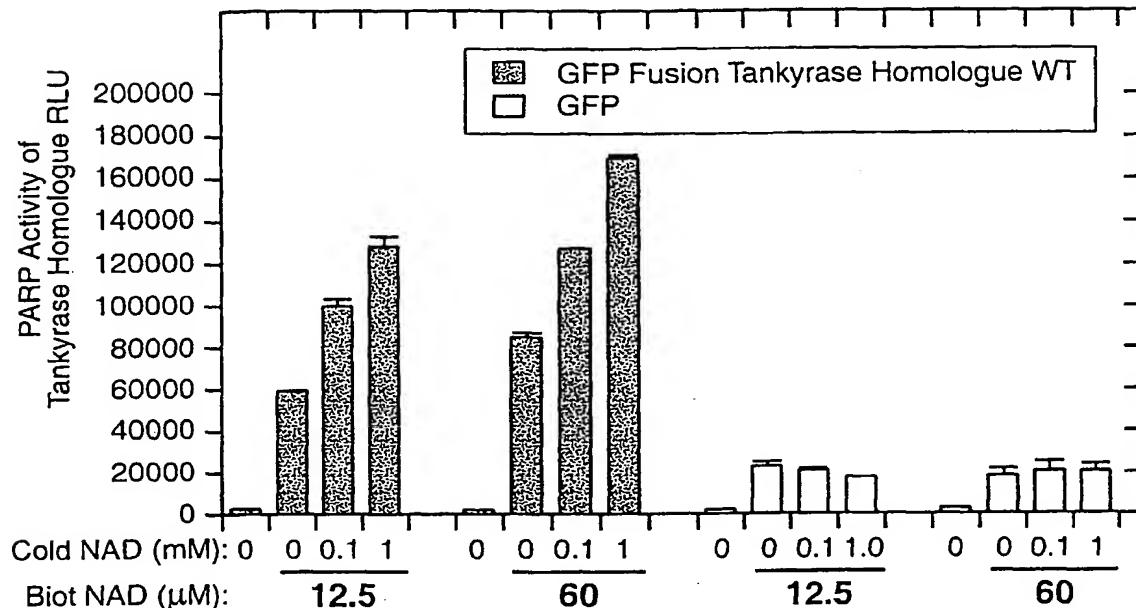
**Procedure for Nonisotopic Detection of Poly-ADP Ribosylation  
Using Anti-GFP mAb-Coated Plates**

Protein lysates from 293T cells normalized by GFP fluorescence and total protein →  
Immobilization of GFP-tankyrase homologue in anti-GFP Coated plates →  
Auto PARP reaction with Biotinylated-NAD in 96 wells →  
Detection of poly ADP ribose chains with Streptavidin-HRP and chemiluminescent substrate



**FIG.\_12**

**Non-Isotopic Plate-Based Detection of TaHo PARP Activity in the Presence of Biotinylated NAD**



**FIG.\_ 13**

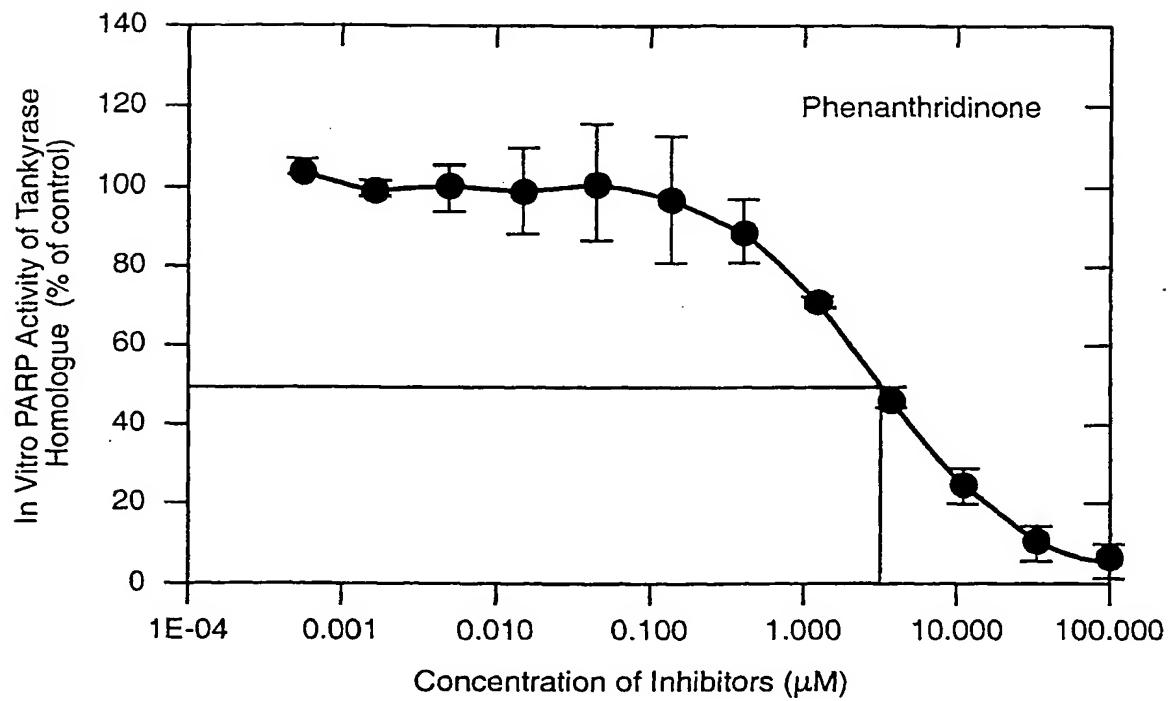
**Comparison of IC<sub>50</sub> Values of the PARP Inhibitors**

	<u>Approximate IC<sub>50</sub> (nM)</u>		<u>hPARP assay IC<sub>50</sub> (nM)</u>		
	<u>TaHo</u>		Rigel	Decker*	Rankin*
3AB	>50 000		5 000	2 000	5 400
6(5H)Phenanthridinone	1 000-2 000		300		
Niacinamide	>50 000		30 000	>>5 000	31 000

\* Decker P et al., Clinical Cancer Research. 1999 May; 5:1169-1172  
 \* Rawkin PW et al., J Biol Chem. 1989 Mar 15;264(8):4312-4317

**FIG.\_ 14**

**Inhibition of Tankyrase Homologue PARP Activity  
by hPARP Inhibitors**



**FIG.\_ 15**

**TH-1: Tankyrase Homologue isoform-1, TH-2: Tankyrase Homologue isoform-2**  
**M (Red): the first methionine in the sequence, Z: stop codon**  
**In this figure, the first methionine in TH-1 sequence is position 1 (iii)**

Taho C terminus deletion mutant ends at position 429 (K) and adds 28 amino acids because of frame shift.

Taho F/L mutant has the mutation at position 871

Taho E/A dc mutant has the mutation at position 948, ends at position 957 (A) and adds 2 amino acids.

<b>TH-1</b> <b>TH-2</b>	<b>RCSARRGAAGGQGAQRGARVGAAGTAPDPVTAGSQ</b> <b>-231</b>	<b>AARALSASSPGLALLLAGPGLLLRLLLAVAAARIMSGRRCAAGGGAACASAAAEEAVE</b> <b>-171</b>	<b>--*GFGRKDVVEYLLQNGA</b> <b>-111</b>	<b>PAARELFEACRNGDVERVKRLVTPEKVNSRDTAGRKSTPLHFAAGFGRKDVVEYLLQNGA</b> <b>-111</b>	<b>Ankyrin repeat</b> <b>Ankyrin repeat</b>
<b>TH-1</b> <b>TH-2</b>	<b>SVQARDGGGLIPLHNACSEGHAEVVNLILRHGADPNARDNWNNTPLHEAAIKGKIDVCIV</b> <b>-51</b>	<b>NVQARDGGGLIPLHNACSEGHAEVVNLILRHGADPNARDNWNNTPLHEAAIKGKIDVCIV</b> <b>-51</b>	<b>Ankyrin repeat</b>	<b>Ankyrin repeat</b>	<b>*TH1 start</b> <b>•TH1 start</b>
<b>TH-1</b> <b>TH-2</b>	<b>LLQHGAEPPTIRNTDGRTALDLADPSAKAVLTGEYKKDELLESARSNEEKMMALLTPINV</b> <b>10</b>	<b>LLQHGAEPPTIRNTDGRTALDLADPSAKAVLTGEYKKDELLESARSNEEKMMALLTPINV</b> <b>10</b>			

## FIG.- 16B

	Ankyrin repeat	Ankyrin repeat	
TH-1 TH-2	NCHASDGRKSTPLHLAAGYNRVKIVQVLLLQHGA DVHAKDKGDLVPLHNACSYGHYEVTTEL 70 NCHASDGRKSTPLHLAAGYNRVKIVQVLLLQHGA DVHAKDKGDLVPLHNACSYGHYEVTTEL 70	Ankyrin repeat	Ankyrin repeat
TH-1 TH-2	LVKHGACVNAMDLWQFTPPLHEAASKNRVECS LLLSSYGA DPTLLNCHNKSAILDAPTPQL 130 LV	Ankyrin repeat	Ankyrin repeat
TH-1	KERLAYEFKGHSLLQAAREADVTRIKKHL SLEMVNFKHPOTHETALHCAASPYPKRKQI 190	Ankyrin repeat	Ankyrin repeat
TH-1	CELLLRKGANINEKTKF FLTPLHVA SEKAHNDVV VEVVVKHEAV YNALDNLGQTSLHRAAY 250	Ankyrin repeat	Ankyrin repeat
TH-1	CGHQQTCRLLLSYGC DPNIISLQGFTALQMGN ENVQQLLQEGISLGN SEADRLQLEAKA 310	Ankyrin repeat	Ankyrin repeat
TH-1	GDVETVKKLCTVQS VNCRDIEGRQSTPL HFAAGYNRVS VVEYLQHGA DVHAKDKGGLVP 370	Ankyrin repeat	Ankyrin repeat
TH-1	LHNACSYGHYEVA ELLVKGAVVN VADLWKFTPL HEAAAKGKYEICK LLLQHGADPTKKN 430	T Ankyrin repeat	Ankyrin repeat Deletion--•
TH-1	GMEILLWILLK MEOIQIFKICL GEMQLCZ RDGNTPLDLYKD GDTDIQDLRGA LLDAAKKGCLAR VKKLSSPDNVNC RDTQGRHSTP 490	Ankyrin repeat	Ankyrin repeat

FIG.-16C

TH-1	LHLAAGGYNLEVAEYLLQHGADVNAQDKGLIPLHNAASYGHVDVAALLIKYNACVNATD Ankyrin repeat	550
TH-1	KWAFTELHEAAQKGRTQLCALLAHGADPTLKNQEQQTPLDLVSADDVSALLTAAMPPSA Ankyrin repeat	610
TH-1	LPSCYKPKQVNLNGVRSPGATA DALSSGPSSPSSLSAASSLDNLSGFSELSVVSSSGTEG Ankyrin repeat	670
TH-1	ASSLEKKE--VPGVDESITQFVRNLGLEHLMDIFEREQITLDVLVEMGHKELKEIGINAY SAM domain	730
TH-1	GHRHKLIKGVVERLISGQQGLNPYLTLNTSGSGTILIDLSPDDKEFQSVEEEQMSTVREHR	790
TH-1	DGGHAGGIFNRYNILKIQKVCNKKILMERYTHRKEVSEENHNHANERMLFHGSPFVNALL PARP domain	850
TH-1	HKGFDERHAYIGGMFGAGIYFAENSSKSNSNQYYGIGGGTGCPVHKDRSCYICHROLLFCR • F→L mutation	910
TH-1	VTLGKSFQFSAMKMAHSPPGHHSVTGRPSVNGLALAEEYVIYRGEOAQAPEYLITYQIMRP • E→A • Deletion.	970
TH-1	EGMVVDG 976	